

Rapid Screening of Methicillin-Resistant *Staphylococcus Aureus* Carrying the *mecC* Gene in a Portuguese Hospital

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ABSTRACT

Background: The discovery of *mecC* gene in MRSA isolates and its report in neighboring Spain and France, advices for monitoring its presence in Portuguese hospitals since changes in local epidemiology can occur quickly. The implementation of rapid procedures based on the automated system VITEK2 allow for simple screening of *mecC* MRSA isolates with the profile of susceptibility to oxacillin and resistance to ceftazidime signaling positive results for detailed analysis.

Methods: This work aims to perform a retrospective study for the presence of suspect *mecC* MRSA in a Portuguese hospital recurring to the two methodologies included in microbiology laboratories routine.

Result: Our results do not suggest the presence of *mecC* MRSA, but highlight the possibility of introducing a simple method in clinical laboratories with high percentage of *S. aureus* isolates.

Conclusion: The implementation of this simple procedure in the laboratory routine is a rapid and economic way to monitoring the presence of *mecC* MRSA.

Keywords: MRSA, *mecC* MRSA, Prevalence, Screening

Introduction

S. aureus is a predominant human pathogen that can cause a diverse range of diseases. Its impact in human health is enhanced by the development of antibiotic resistances, mainly through Methicillin Resistant *S. aureus* (MRSA) [1].

MRSA is resistant to almost all β -lactam antibiotics, and it is known that its resistance is caused by the production of a penicillin-binding protein (PBP), named PBP2A, that reduces the binding affinities to β -lactam antibiotics. PBP2A is encoded by the gene *mecA* located in MRSA chromosome [2].

The identification of MRSA in diagnostic microbiology laboratories can be achieved by a variety of methods, including antimicrobial susceptibility testing, detection of PBP2a by latex agglutination tests and molecular detection of the *mecA* gene [3].

In 2011, a new *mecA* gene homolog, *mecALGA251*, was found in isolates from both humans and animals. The International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements renamed it to *mecC* [4]. The *mecC* gene shares 69%

nucleotide homology with *mecA* and was found in a novel SCC element designated as SCCmec type XI [4].

MRSA isolates harboring the *mecC* gene have been reported in several European countries mainly from humans who had contact with livestock, and/or wild and domestic animals, emphasizing that contact with animals poses a zoonotic risk and that *mecC* MRSA can be transmitted between species and consequently could be regarded as a LA-MRSA [1].

The discovery of *mecC* MRSA is of concern for microbiology laboratories since these isolates originate negative results, both by latex agglutination test and by the PCR assay for *mecA* [1, 3]. Routine diagnostic tests do not enable the identification of *mecC* gene, which still requires confirmation using PCR assays that are currently available only at reference laboratories.

The cultural and geographical proximity of France and Spain allied with close to 50% for over 10 years of MRSA prevalence in Portuguese hospitals, one of the highest in Europe [5], highlights the urgency to implement routine laboratory testing methods to rapidly detect and treat these emerging strains.

mecC MRSA isolates are susceptible to oxacillin but resistant to cefoxitin, when tested using Staph AST cards on Vitek2 automated antimicrobial susceptibility testing system (BioMérieux, France)^[3] a profile that differs from other MRSA strains. This simple procedure enables the laboratory to suspect of *mecC* MRSA presence. By performing it routinely, laboratory teams and physician can monitor changes in the hospital *mecC* MRSA distribution and prevalence over time^[3].

Materials and Methods

Aims and Study Design: This work aims to perform a retrospective study for the presence of suspect *mecC* MRSA in a Portuguese hospital recurring to the two methodologies (PBP2a and Vitek2) included in microbiology laboratories routine.

Study Population and Data Collection: Based on database from the microbiology laboratory of Centro Hospitalar Baixo Vouga, Aveiro, Portugal, a retrospective analysis of 835 *S. aureus* isolates, between 2014 and 2016 was performed. Only one isolate per patient was included. Isolates were collected from various clinical sources, including respiratory tract ($n = 192$), blood ($n =$

190), wounds ($n = 324$), urine ($n = 83$) and others ($n = 46$). Patient demographic characteristics and clinical data were obtained from the medical records.

Laboratory Methods: Data of oxacillin and cefoxitin obtained with automated system (Vitek2-Biomerieux) was analyzed. To test the inclusion of this procedure in the day-to-day activities of the clinical laboratory a sample of 84 MRSA strains collected from Medicine department during 2014 were retested for the PBP2a test (Alere). A positive control of *mecC* MRSA – negative to PBP2a, susceptible to oxacillin and resistant to cefoxitin – was included in this analysis to procedure validation^[1].

Result

Between 2014 and 2016 a total of 835 *S. aureus* isolates were identified by the microbiology laboratory with 409 classified as MRSA (Table 1). A sample of 84 MRSA from 2014 was retested for PBP2a. All 409 MRSA were resistant to both oxacillin and cefoxitin (Table 2) and all 89 retested isolates were positive to PBP2a test. The positive control for *mecC* MRSA^[1, 3] originated a negative result on the PBP2a test and was susceptible to oxacillin and resistant to cefoxitin when tested with AST619 Staphy cards on automated system Vitek2.

Table 1: Characterization of *S. aureus* isolates between 2014 and 2016, in Centro Hospitalar Baixo Vouga, Aveiro, Portugal.

Year of analysis	2014	2015	2016
Isolates (n)	291	249	295
Age (Mean \pm SD)	64,9 \pm 23,5	65,2 \pm 22,2	71,7 \pm 23,7
Male sex, n (%)	178	146	172
Clinical Source (%)			
Respiratory tract	81	44	67
Blood	62	62	66
Wounds	108	107	109
Urine	27	26	30
Other sources	13	10	23

Table 2: Susceptibility profile on automated method (Vitek2, 619 AST card)

	Number of isolates (% of year total)			
	Control	2014	2015	2016
<i>S. aureus</i> isolates		291 (100%)	249 (100%)	295 (100%)
MRSA isolates		176 (60,5%)	121 (48,6%)	112 (37,9%)
Oxacillin/Cefoxitin Profile	-	176	121	112
R/R	-	-	-	-
S/S	Positive	-	-	-
S/R				
PBP2a test*	Negative	-	-	-

Discussion

Based in the 2015 ECDC report, Portugal is the third European country with the highest prevalence of MRSA (46,8%), nevertheless is one of the countries that shows a significant decreasing trend [6]. In the last decades, MRSA infections were limited to hospitals, but the exponential emergence of community-associated MRSA infections shaped the epidemiology of *S. aureus* leading to the replaced of hospital-associated MRSA (HA-MRSA) clones by community-associated MRSA [7]. Between 2009-2010 Tavares A, *et al.* [8] studied a total of 1,487 *S. aureus* isolates collected from 16 Portuguese healthcare institutions located in different geographic regions of Portugal, reporting that MRSA clones present in the community were clones typically found in hospitals. This signals Portugal as a region where the transfer of strain between setting can be recognized, valorizing potential risks for public health of a third epidemiological form of MRSA, livestock-associated MRSA (LA-MRSA) [1].

In humans, *mecC* MRSA was isolated in hospital patients from Slovenia [9], Germany [10], Republic of Ireland [11], Denmark [12], Spain [13] [14] and France [15]. Garcia-Garrote *et al.* [14] reported the first *mecC* MRSA fatal human case in Spain, a patient with sepsis who died in the emergency department. In the same study *mecC* MRSA was reported in 6 other human cases, predominantly in skin and soft tissue infections. In France, Laurent, *et al.* [15] described the presence of *mecC* MRSA in joint infections in humans and clustered animal cases, 2 cows with clinical mastitis, demonstrates the ability of such strains to cause clustered cases.

Studies in UK [12], France [15], Sweden [16] and Finland [17] confirmed *mecC* MRSA wide geographic range and shown frequent presence in dairy cattle. A study in Germany found it in companion animals [18]. These findings suggest that dairy cattle, companion animals and wild animal, might work as reservoirs of infection and could share *mecC* with other animals, when they coexist in the same habitat.

Our results do not suggest the presence of *mecC* MRSA, but highlight the possibility of introducing a simple method in clinical laboratories with high percentage of *S. aureus* isolates, allowing a routine and rapid screen of the presence of *mecC* MRSA, contributing for monitor changes in *mecC* MRSA prevalence over time. This type of method can rapidly and easily be introduced in the majority of clinical laboratories and performed in all MRSA isolates. Although the overall prevalence of *mecC* MRSA is still very low (0,009%), higher in animals (0,1%) than in humans (0,004%), it is important to evaluate clinically *mecC* MRSA presence, to clarify its epidemiology [19]. In fact, the clinical of *mecC* MRSA is not fully determined and study

alternatives that can contribute to understand its impact is fundamental. Screening the presence of *mecC* MRSA with routine diagnostic tests is a useful and important procedure, specially in regions with high prevalence of MRSA, like Portugal. Currently, *mecC* MRSA do not constitute a risk for public health but changes in local epidemiology can occur quickly, especially when the strain is already present in neighboring countries, such as Spain.

Conclusion

This work showed that, a minimal effort and investment in health professionals training is sufficient to keep a clinical laboratory team in alert to the emerge of *mecC* MRSA. Implementing this strategy will allow, in the event of positive results for *mecC* MRSA, to a rapid shift to more advance molecular methods, as PCR, avoiding future false negatives for MRSA, which ultimately leads to better treatment and management of the affected population, with better clinical outcomes expectation.

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